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Abstract

Between May 2006 and January 2007, blood samples and ticks were randomly collected from 220 nomadic animals from Filtu and Dollo Odo districts, Libaan zone, in the Somali Region of Ethiopia. Overall, 81.5% cattle, 98.2% camels, 53.4% goats and 61.1% sheep were infested by Ixodid ticks. Collected ticks (n=1,036) were identified as *Rhipicephalus pulchellus* (40.1%), *R. pravus* (25.8%), *Amblyomma gemma* (9.4%), *Hyalomma rufipes* (13.3%), *H. truncatum* (2.8%), *H. impeltatum* (1.2%) and *H. dromedarii* (0.5%); immature stages (6.1%) belonged to the genera *Rhipicephalus* and *Amblyomma*. Tick infestation burden was evaluated by the Tick Abundance Score method on 57 animals from Dollo Odo in August 2006, and it was significantly higher in cattle and camels than in small ruminants (p<0.001).

Reverse Line Blot Hybridisation was applied to detect *Theileria*, *Babesia*, *Ehrlichia* and *Anaplasma* spp.. Five out of 50 blood samples from Filtu, 4 from cattle and, surprisingly, one from a camel, were positive for *Theileria mutans* and 2 from cattle for *Theileria velifera*. Adult ticks (n=104) from both districts were tested and *A. gemma* from cattle were positive to *T. velifera* (1) and *Ehrlichia ruminantium* (5 samples). Positive *E. ruminantium* samples were also tested by PCR targeting pCS20 and 16S rRNA genes and submitted to DNA sequencing. The phylogenetic reconstruction of pCS20 fragment showed the presence of the Somali region sequences in the East-South African group.

Our results are the first available on ticks and selected tick-borne diseases from the Somali region of Ethiopia and could be used as preliminary information for planning sustainable control strategies for tick and tick-borne pathogens in the study area and in neighbouring areas with similar socio-ecological features.

Keywords: Ixodid ticks, nomadic herds, Somali Region, Ethiopia, RLB, *Ehrlichia ruminantium*, *Theileria* spp.

INTRODUCTION

Livestock in Ethiopia plays a vital role in the livelihoods of the rural communities, constituting a major source of income (~16% of the total gross domestic product –GDP– and over 30% of the total agricultural GDP). According to the Agricultural Sample Survey of 2009 by the Ethiopian Central statistics agency, Ethiopia has one of the largest livestock inventories in Africa, including more than 49 million cattle, 47 million small ruminants, 7.6 million equines, 760,000 camels and 42 million chickens (Anon. 2009). The livestock sector supplies animal protein, manure and traction power. Of the total, it is estimated that the pastoral nomadic sector keeps 40 % of the cattle, 75% of the goats, 25% of the sheep, and 100 percent of the camels (Anon. 2003).

Infectious and parasitic diseases of animals are thus considered of paramount importance, especially within pastoralist communities. Animal health problems are generally exacerbated by drought, concentration of livestock at watering points and pasture grounds, and scarce access to veterinary services in many parts of the country (Anon. 2001). Ticks and tick-borne diseases (TBD) cause considerable losses to the livestock economy in Ethiopia, together with trypanosomosis and endoparasitism (Pegram et al. 1981). Tick bites damage skins and hides, predispose to infections and cause immunodepression to animals (Abera et al. 2010, Chanie et al. 2010, Mulugeta et al. 2010). Moreover, ticks are vectors of diseases such as ehrlichiosis, anaplasmosis and babesosis. These diseases are reported in the country, even if they do not appear to be as important as other livestock diseases (Pegram et al. 1981), probably thanks to the natural resistance of the local breeds.

Morel (1980) reported more than 60 tick species infesting wild and domestic animals in Ethiopia. However, bibliographic information on the geographical distribution of the tick species infesting domestic animals as well as their impact on livestock production in the Somali Region is not available. In the Somali Region, the eastern region of Ethiopia, 83% of the population lives in rural areas with a pastoral livelihood system, rearing camels, cattle and small ruminants. People are heavily dependent on livestock and livestock products for food and income.

The goal of our study was to identify the most common tick species and TBD in the Libaan administrative zone of Somali Region, in order to contribute towards planning and implementing effective tick control strategies. Our work was conducted within the framework of a research and development project aimed at providing basic health services to the local nomadic populations and monitoring major livestock health problems in the study area.

MATERIALS AND METHODS

Study Area

The Somali Region consists of 9 administrative zones, 44 *wereda* (administrative districts), and 67 urban settlements. The region is overwhelmingly rural and the level of urbanisation is low (14.3%). Its population was calculated at 3.5 million in 1997, mainly composed of Ethiopian Somalis (96%) (Anon. 1998).

Our research was conducted in 23 rural villages in Filtu e Dollo Odo districts of Libaan administrative zone, located from 03°57'59'' to 5°19'07''N and from 39°55'59'' to 42°03'25''E. Most of the study area is lowland plain, ranging from 1,300 m in the higher area around Filtu and decreasing to around 200 m above sea level at Dollo Odo. The climate is arid, with a mean annual temperature of 27°C, mean rainfall less than 450mm, and low humidity. Four seasons are present: a rainy spring from April to June (called '*Gu*'), a dry summer from July to September ('*Hagaa*'), a short rainy autumn in October-November ('*Dayr*'), and a long dry season from December to March ('*Jjilaal*').

The pastoral area is characterized by three types of vegetation: scattered tall trees, shrubs, grassland. Camels and goats are concentrated in zones with thorny tall trees and shrubs, while cattle and sheep graze in the grasslands. Rains in the wet season contribute to pasture availability and surface water, thus allowing most herds to remain near home. During the dry season, when local pastures become

depleted, herds are split and strong animals migrate over great distances. The animals are kept under traditional management and are bred for meat and milk production. The most common livestock bred are: Small East African Zebu cattle and their crosses with the Borana breed, Blackheaded Somali sheep, Galla and Somali goats, and camels. Donkeys and camels are used as beasts of burden (Anon. 2002).

Blood and tick collection

A health monitoring of livestock was conducted in 12 villages in Filtu from May to July 2006, while 11 villages in Dollo Odo were studied in August, November and December 2006, and in January 2007. Sampling was strongly influenced by field conditions: availability of the livestock keeper, time and logistic constraints, tractability of the animals. Animal blood was collected and stocked in LongMire Buffer (Randi et al., 2002), then stored at 4°C until DNA extraction. In each village, a sample of ticks was collected from 7-10 randomly selected animals, during the time needed for blood sampling and clinical examination (around 3 minutes). Overall, 26 cattle, 40 camels, 27 goats and 7 sheep were inspected for ticks in Filtu (n=100 animals). In Dollo Odo, 120 animals were examined: 39 cattle, 17 camels, 31 goats, 11 sheep; furthermore, 22 unknown animals were also studied in Dollo Odo, of which details on samples collection (field sheets) were lost. Considering the logistic constraints and the scarcity of information from the study area, we deemed it important to include anyway the data on the ticks collected from these 22 animals.

The ticks from each animal were preserved in separate labelled vials containing 70% ethanol, and subsequently counted and identified to species level using a stereomicroscope and identification keys (Walker et al. 2000, 2003). Nymphs were classified at genus level.

Tick Abundance Score (TAS) was attributed to a sample of animals in seven Dollo Odo villages in August 2007 to assess the tick infestation burden, according to the protocol described by Mooring and McKenzie (1995), partially modified. Each animal was visually checked by the same operator

for the presence of ticks in 3 pre-defined body regions (head-neck; thorax-abdomen; perineum-tail), and TAS (0 = no ticks; 1 = 1-20 ticks; 2 = 20-60 ticks; 3 >60 ticks) was attributed to the respective body region. Animals were not subjected to acaricide treatments during the study period.

Molecular detection of tick-borne pathogens

Blood samples and adult ticks were analyzed by PCR and Reverse Line Blot Hybridization (RLB) to detect tick-borne pathogens. Fifty blood samples were screened, all collected in May 2006 from 50 animals (17 cattle, 22 camels and 11 goats) belonging to 6 villages in Filtu district. No blood samples from Dollo Odo district nor samples collected in other periods in Filtu were tested, due to limited economic resources. Tested ticks (n=104) were randomly chosen from all villages and animal species, giving priority to ticks known to be vectors of diseases (*Amblyomma gemma, Riphicephalus pulchellus*). Part of the ticks were individually tested (n=44) and part were pooled and processed in batches of 2–3 tick specimens belonging to the same animal, species and sex (n=60 in 23 pools). Tested ticks belonged to both Filtu (n=49) and Dollo Odo (n=55) districts.

DNA was extracted from blood and ticks by DNeasy Blood and Tissue kit (QIAGEN Valencia, CA, USA). A number of negative controls (distilled water) were run alongside the samples in random order throughout the experiments. PCR and RLB, using the TBD-RLB membrane (ISOGEN Life Science, The Netherlands), were performed as described by Bekker et al. (2002) and Gubbels et al. (1999). *Ehrlichia ruminantium* positive samples were also subjected to PCR to amplify a 280 bp fragment of pCS20 gene by using a nested assay (Faburay et al. 2007) and a 500bp fragment of the small subunit ribosomal RNA gene (16S rRNA; Gramley et al. 1999).

Phylogenetic analysis

Sequences obtained from *E. ruminantium* positive samples were aligned with all known *E. ruminantium* sequences available on the GenBank database using the program ClustalW (Thompson et al. 1997). The aligned sequences were imported into the computer program PAUP*

ver. 4.0b10 (Swofford 2003). The model of molecular evolution was estimated using a hierarchical likelihood ratio test approach and the Akaike information criterion (Akaike 1973) implemented in the computer program ModelTest ver. 3.7 (Posada and Crandall 2001). Bayesian methods implemented in the computer program MrBayes ver. 3.1.1 (Ronquist and Huelsenbeck 2003), were used to draw phylogenetic trees and assess statistical support for clades. In detail, a Markov chain Monte Carlo search for 1,000,000 generations using two runs with four chains (temperature = 0.05) was performed, and results were represented as a 50% majority rule consensus tree. The sequence identity comparisons were made by the Nei-Gojobori method (Nei and Gojobori 1986).

Statistic analysis.

Prevalence of tick infestation for vertebrate hosts and tick species was calculated with exact binomial 95% confidence intervals (CI). The Fisher Exact Test was used to study the association among categorical variables and the Kruskal-Wallis test to study the differences of TAS in animal species. Those samples which were not identified by host species (n = 22 from Dollo Odo) were excluded from the analysis on the tick-host association.

The R software (http://www.R-project.org) was used to perform the statistical analyses. Prevalence of infection by tick-borne pathogens in tick pools were calculated by using the Pooled Prevalence Calculator (Sergeant 2009), with a 95% confidence level.

RESULTS

Overall, 73.6% of the examined animals were found infested by ticks in the study area. A significant difference in infestation prevalence was registered among species (p<0.001), with camels being the most parasitized animals (Table 1). A total of 1,036 ticks were collected (973 adults and 63 nymphs). Most ticks (71.6%, n=742) were collected in Filtu villages, in the period May-July 2006.

Adult ticks were identified as *Rhipicephalus pulchellus* Gerstäcker, 1873 (42.7% of adults; n=416), *Rhipicephalus pravus* Dönitz, 1910 (27.4%; n=267), *Amblyomma gemma* Dönitz, 1910 (10.1%; n=98), *Hyalomma rufipes* Koch 1844 (14.2%; n=138), *Hyalomma truncatum* Koch, 1844 (3.0%; n=29), *Hyalomma impeltatum* Schulze & Schlottke, 1930 (1.2%; n=12), *Hyalomma dromedarii* Koch, 1844 (0.5%; n=5). Nymphs were classified as *A. gemma* (6.3% of nymphs; n=4), *R. pravus* (36.5%; n=23), *R. pulchellus* (28.6%; n=18). Four *Hyalomma* spp. adults and 18 *Rhipicephalus* spp. nymphs could only be identified at genus level as they were damaged.

Rhipicephalus nymphs were mostly collected in July and January (dry periods), while adults were more abundant during the rainy months (May-June and November). As regards other adult ticks, *A. gemma* were abundant in May, and *Hyalomma* spp. in January, August and November.

The tick species collected on different hosts are summarised in Figure 1. Cattle were especially infested by *R. pulchellus* (57.5%), followed by *H. rufipes* (16.3%), *R. pravus* (9.6%) and *A. gemma* (9.6%). *R. pulchellus* (35.4%), *R. pravus* (31.4%), *A. gemma* (14.5%) and *H. rufipes* (14.1%) infested camels. *R. pravus* was the most common species on goats (62.6%) and sheep (54.5%), followed by *R. pulchellus* (35.9% and 36.4% respectively).

TAS was attributed to 57 animals (31 cows, 8 camels, 16 goats and 2 sheep) in 7 villages from Dollo Odo district. A significant difference in the score was detected among host species ($p < 0.001$). Cattle and camels were more infested than small ruminants (Figure 2).

By PCR/RLB, 5 of the 50 blood samples (10%; 95%CI: 3.3, 21.8)were positive for *Theileria mutans*: 4 cows and, surprisingly, 1 camel. Two of these cows were also positive for *Theileria velifera* (4%; 95%CI: 0.5, 13.7).

Tested ticks (n=104) were collected from cattle (n=54), camels (n=35), goats (n=13) and sheep (n=2) and belonged to the following species: *A. gemma* (n=24), *R. pulchellus* (n=21), *R. pravus*

(n=3), *H. rufipes* (n=13), *H. truncatum* (n=4), *H. impeltatum* (n=2). *T. velifera* was detected in one *A. gemma*; this tick was collected from cattle in Dollo Odo

(0.96%; 95%CI: 0.0, 2.8). Five of the tick samples (3.4%; 95%CI: 1.2, 7.1) gave a signal to the *E. ruminantium* probe on the RLB membrane; all of them were *A. gemma* collected on cattle, 3 from Filtu (one single male, one pool of 3 males and one pool of 3 females) and 2 from Dollo Odo (two single *A. gemma*, one male and one female). The result was confirmed by pCS20 PCR and four samples were successfully sequenced. Three of the sequences were identical, so only one (Er20; GenBank accession no.: GU797236) was included in the phylogenetic analysis, together with the fourth sequence (Er31, GenBank accession no.: GU644448). The phylogenetic tree derived from comparison of the partial 280 bp pCS20 nucleotide sequences is presented in Figure 3. This phylogenetic reconstruction showed the presence of our new Somali Region sequences in the group of East-South Africa sequences, precisely in a cluster with the Gedaref strain derived from Sudan (GenBank accession no.: AB218277). The comparison of the percentage of identical nucleotides among the inferred pCS20 sequences revealed an identity to the Gedaref strain of 100% for Er20 and 99.5% for Er31. Our sequences also clustered to the strains Gardel from the Caribbean and Welgevonden, Vosloo and Ball3 from South Africa. Sequence similarities among members of the East-South Africa group were 98.84%, while sequences from the West Africa cluster showed a 100% similarity. Sequence similarities of the Kumm2/ErPMtb group and the other *E. ruminantium* sequences ranged from 87.36 to 88.75% .

Only one *E. ruminantium* 16S rRNA gene positive sample was successfully sequenced and showed a similarity of 100% with Gardel, Kiswani, Welgevonden, Umm Banein *E. ruminantium* strains, thus confirming the previous findings.

DISCUSSION

Our study provides new information on tick fauna, on host distribution of ticks and on tick-borne pathogens in the Somali Region of Ethiopia, with special reference to the Libaan zone. Logistic factors strongly conditioned our tick collection, which was not systematic and comprised different study sites in different periods, being mainly due to the nomadic livestock keeping system in the study area.

We detected *Erlichia ruminantium* in our tick samples and, to the best of our knowledge, this is the first report of the pathogen in *Amblyomma gemma* field ticks collected on livestock. This *Amblyomma* species has been proven to be capable of transmitting heartwater in the laboratory (Walker and Olwage 1987), and, experimentally, from wild ruminants to livestock (Wesonga et al. 2001). However, according to literature, *A. gemma* has not been implicated in outbreaks of the disease (Walker and Olwage 1987). Wesonga et al. (1993) suggested anyway the concrete possibility that *A. gemma* could acquire the pathogen in an endemic area and then transmit the infection to susceptible livestock under natural conditions. This possibility has to be further investigated as other related species (*A. lepidum, A. astrion* and *A. pomposum*) are known to be involved as vectors in outbreaks of heartwater, in addition to the most important competent vectors, *A. variegatum* and *A. hebraeum* (Walker and Olwage 1987).

Phylogenetic analysis of the pCS20 sequences of our *E. ruminantium* positive samples indicate a high similarity with strains detected in Sudan and locate our sequences in the Southern-Central Africa and Caribbean clade. As previously reported, our analyses highlighted the ability of pCS20 gene of *E. ruminantium* to reflect the geographic distribution of the strains. This result confirms the greater variability of East South Africa group compared with the more conserved West Africa clade (Allsopp et al. 2003, 2007; Van Heerden et al. 2001). This variability could be justified by an extensive animal trade present within the East and South African regions. A PCR targeting

the16srRNA gene was also performed to confirm *E. ruminantium* classification; indeed, this is considered the gene of election to characterize this bacterium, based on >99% identity percentage (Allsopp 2010). Although only one of our samples was successfully sequenced, our results confirmed its location in the *E. ruminantium* cluster.

The low *E. ruminantium* infection prevalence detected could be partly due to the diagnostic techniques used. Indeed, even if RLB is a very convenient tool for the simultaneous screening of samples for several pathogens, its sensibility has been shown to be lower as compared to other techniques for the detection of *E. ruminantium* (Faburay et al. 2007). However, livestock keepers in the area do not report mortality associated to tick-borne diseases, in particular clinical signs attributable to cowdriosis have not been described. Thus a limited circulation of *E. ruminantium* in the study area seems to be consistent with the low infection prevalence detected in our samples.

Regarding other tick-borne agents, we detected *Theileria mutans* and *T. velifera* in blood and tick samples. The circulation of *Theileria* spp. in Ethiopia was already shown by Solomon et al. (1998), who reported a 30.9% seropositivity to *T. mutans* in cattle from the Yabelo district. *T. mutans* infection can result in mild clinical signs, but pathogenic strains in eastern Africa cause severe anemia, icterus and sometimes death (Lawrence and Williamson 2004a). *T. velifera* is considered not pathogenic for cattle (Lawrence and Williamson, 2004b), so its field control is not generally necessary.

Theileria mutans infection has not been reported before in the camel. During this study we found the blood of one camel positive for *T. mutans* by PCR/RLB. This finding should be further explored.

Results on tick species are consistent with previous reports from arid and semi-arid areas of Ethiopia. Indeed, Regassa (2001) identified *R. pulchellus* as the main tick infesting cattle, followed by *R. pravus* and *A. gemma* in the Borana Province. In Errer Valley, Eastern Ethiopia, *Amblyomma*

variegatum (41%), *R. pulchellus* (25.6%) and *A. gemma* (13%) were reported as the major species infesting domestic animals (Tafesse, 1996), with *R. pulchellus* being the most abundant on camels (Zeleke and Bekele 2004). In a study conducted in the southern rangelands of Ethiopia (Yabelo district) by Solomon et al (1998), *R. pulchellus* was the most abundant species on cattle (82.4%), followed by *A. variegatum, Rhipicephalus* (*Boophilus*) *decoloratus* and *A. gemma*. On the contrary, *R. (Boophilus) decoloratus* was by far the most prevalent tick in goats and sheep in Miesso district of Oromia Region, characterized by a wetter and colder climate (Abunna et al. 2009).

As Filtu and Dollo Odo districts were studied in different seasons, it was not possible to compare the infestation prevalence and tick species collected in the two areas. Moreover, due to the opportunistic nature of our sample, it was not possible to analyze the tick seasonal dynamics and tick burdens. However, the TAS assessment gave us an estimate of the real infestation burdens on animals. Camels were the most infested animals and also had the highest prevalence of infestation, indeed almost all examined animals (98.2%) were found infested by ticks.

Overall, adult parasites were collected during the whole study period and most tick species infested all hosts. In our study area, animal herds are generally mixed at grazing places, hence we hypothesise that mixed grazing may diminish host specificity of some ticks species. For example, *R. pravus* was collected on camels in particular, although this tick reportedly prefers to feed on cattle (Walker et al. 2000). *R. pravus* is normally associated with *A. gemma* in dryland areas (Matthysse and Colbo 1987, Walker et al. 2003) and this feature is consistent with our results. This tick species was the most abundant together with *R. pulchellus*, a species considered one of the commonest ticks of domestic livestock in the Horn of Africa.

Data collected in the study area –although the first available- might not be enough to draw conclusions/suggestions as regards implementation of strategies for ticks and TBD control. Considering the low tick burden observed and the low prevalence of infection by tick-borne pathogens, it is likely that no control measures are needed under the present circumstances. It is

likely that a certain degree of endemic stability for ticks and TBD is present, and –even if not yet completely achieved- it should be maintained and further strengthened. This could reduce the use of chemical acaricide to a minimum, and can make tick and TBD control sustainable and ecologically sound. However, climate-driven changes and modifications in land use and management could require in future the implementation of an integrated strategy for tick and TBD control (e.g. *ad hoc* or threshold regime).

CONFLICT OF INTEREST STATEMENT

None of the authors of this study have any financial or personal relationships with other people or organisations that could have inappropriately influenced this work.

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Figure 1. Tick species by hosts collected in Filtu and Dollo Odo districts, Somali Region, Ethiopia.
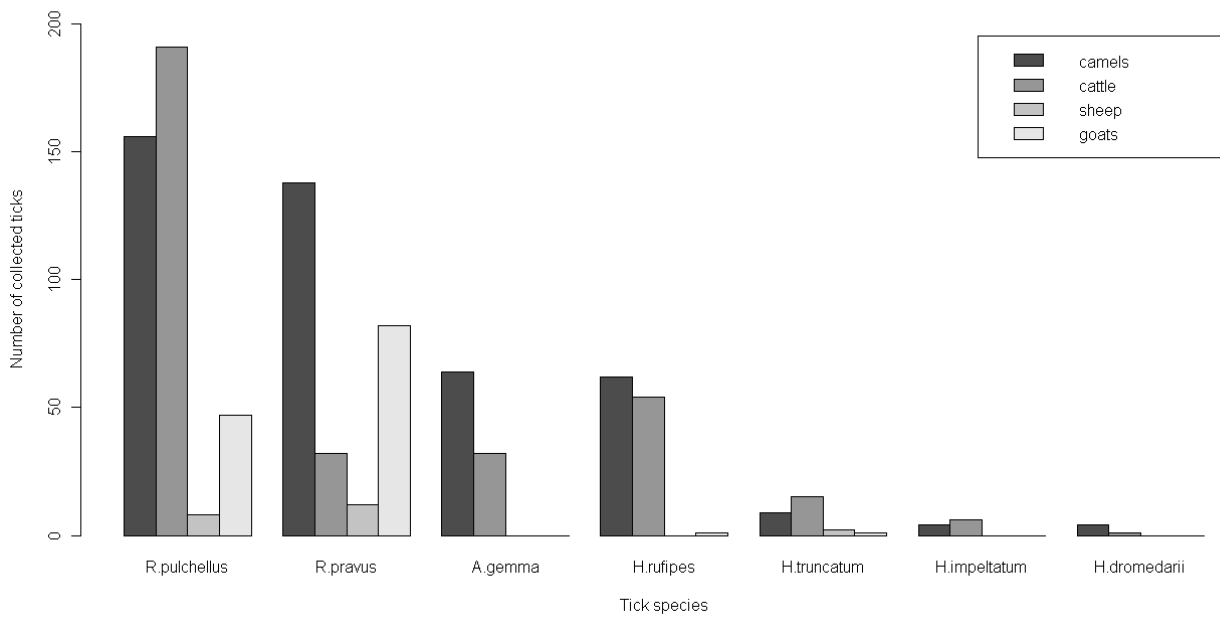

Figure 2. Boxplot of the TAS attributed to host species in Dollo Odo district, August 2007 (0 = no ticks; 1 = 1-20 ticks; 2 = 20-60 ticks; 3 >60 ticks).
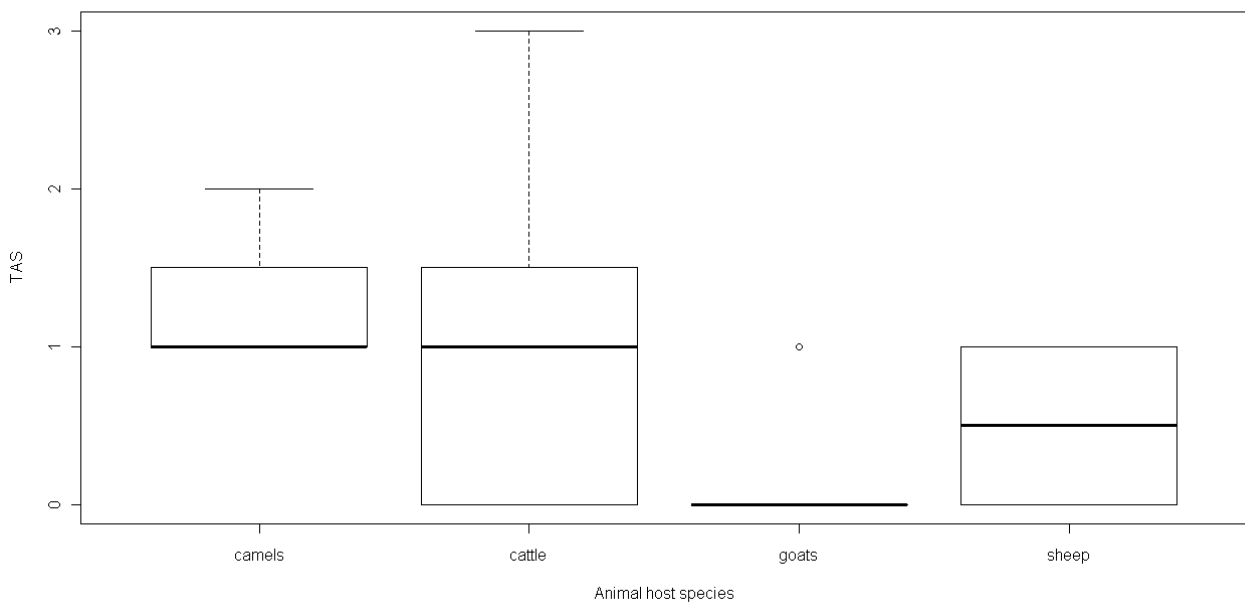
Figure 3. Phylogenetic tree of new *E. ruminantium* from Filtu and Dollo Odo districts and NCBI reference sequences (see text for details), based on the comparison of the partial 280 bp pCS20 nucleotide sequences. Posterior probability values are reported below branches. New Somali Region sequences are underlined. Bar: 0.01 substitutions per site. Legend: W-A West Africa, E/S-A: East and South Africa.
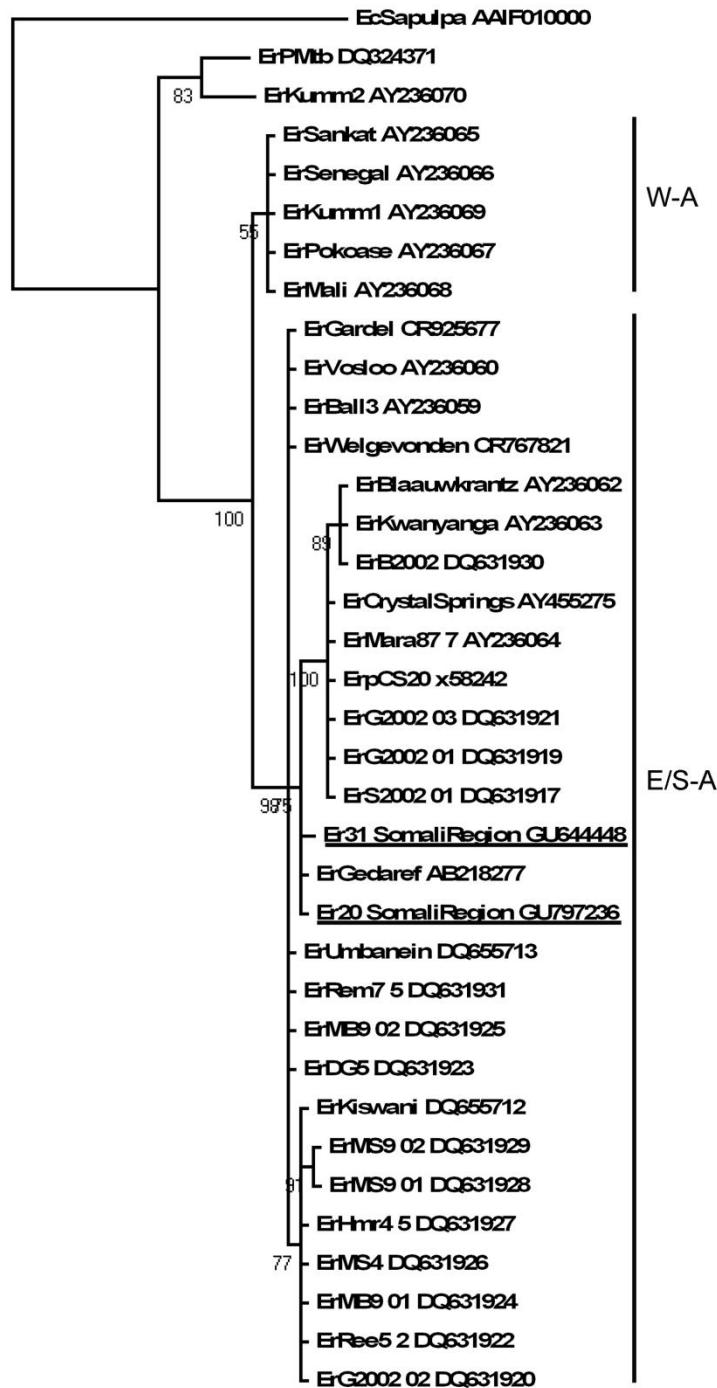
Table 1. Number of animals examined for the presence of ticks and prevalence of infestation (with number of positives and 95% confidence intervals) in Filtu and Dollo Odo districts, Somali Region, Ethiopia.

Animal species	N of examined animals	Infestation prevalence: % (n, 95% CI)
Cattle	65	81.5 (53, 70.0-90.1)
Camels	57	98.2 (56, 90.6-99.9)
Goats	58	53.4 (31, 39.9-66.7)
Sheep	18	61.1 (11, 35.7-82.7)
Total	198	76.3 (151, 69.7-82.0)